2 copies of Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo-Report of the in-house investigations of **Sodium Sulfite** in the developing chicken embryo 4/15/75

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

F27

TO : Mr. Alan Spiher
GRAS Review Branch, HFF-335

DATE: April 15, 1975

THRU : Dr. Herbert Blumenthal, Acting
Division of Toxicology, HFF-150

FROM : M. Jacqueline Verrett, Ph. D.
Reproductive Physiology Branch, HFF-157

M. Jacqueline Verrett

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo.

Attached is the report of the in-house investigations of Sodium Sulfite in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Sodium Sulfite

Protocol:

Sodium Sulfite (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one-hundred eggs per level was reached for all levels allowing some hatch. Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in tables 1 through 4 for each of the four conditions of test.

Column 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and the untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Air cell treatment at 0 and 96 hours resulted in LD_{50}'s of 1.035 and 0.833 mg/egg, respectively. Yolk treatment at 0 hours showed significant toxicity above the solvent but the slope of the line was not significantly different from zero (p=0.05). The slope of the line was negative for yolk treatment at 96 hours.

Scattered abnormalities were observed for all four conditions of test, but there were no serious structural abnormalities (column 6) that were different from the solvent treated or untreated control eggs in either nature or number. Sodium sulfite displayed no teratogenicity under the test conditions employed.

1. Sodium Sulfite, Lot # H028, Allied Chemical Co., Morristown, N.J.

2. McLaughlin, J., Jr., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O. G., (1963) Toxicol. Appl. Pharmacol. 5, 760-770.

3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002 - 1006

4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

Table I

Sodium Sulfite

Air Cell @ 0 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.000	100.00	160	93.12*	6.87	1.25
2.500	50.000	160	80.62*	14.37*	5.00
1.250	25.000	120	65.00*	6.66	1.66
0.500	10.000	160	50.00*	10.00*	4.37
0.250	5,000	120	38.33	2.50	0.00
Water		164	34.14	1.82	0.60
Controls		492	26.01	1.01	0.20

** LD_{50} 20,7109 mg/kg (1.0355 mg/egg)

* Significantly different from solvent p=0.05

Table II

Sodium Sulfite

Air Cell at 96 Hours

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
2.500	50.000	110	100.00*	0.00	0.00
1.250	25.000	110	88.18 *	0.90	0.00
0.6250	12.500	109	44.95	2.75	0.91
0.250	5.000	109	41.28	4.58	3.66
0.1250	2.500	110	28.18	1.81	0.00
Water Controls		95	36.84	1.05	1.05
		492	26.01	1.01	0.20

** LD_{50} 16.6644 mg/kg (0.8332 mg/egg)

* Significantly different from solvent p=0.05

Table III

Sodium Sulfite

Yolk @ 0 Hours

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.000	200.000	15	93.33*	0.00	0.00
5.000	100.000	120	81.66*	2.50	1.66
2.500	50.000	120	81.66*	0.83	0.00
1.250	25.000	120	74.16*	0.00	0.00
0.500	10.000	120	78.33*	1.66	0.83
0.250	5.000	120	76.66*	0.83	0.83
Water		90	37.77	1.11	1.11
Controls		492	26.01	1.01	0.20

** Slope not significantly different from zero (p=0.05)

* Significantly different from solvent p=0.05

Table IV

Sodium Sulfite
Yolk @ 96 Hours

Dose		Number of Eggs	**Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
2.500	50.000	110	57.27*	5.45	0.90
1.250	25.000	110	51.81*	1.81	0.90
0.0250	12.500	110	58.18*	0.90	0.00
0.250	5.000	110	55.45*	2.72	0.00
0.1250		110	55.45*	0.90	0.00
Water		90	34.44	0.00	0.00
Controls		492	26.01	1.01	0.20

** Slope is negative

* Significantly different from solvent $p \leq 0.05$

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